molecular diagnostic test for quantitative determination of breast cancer biomarkers ER, PR, HER2, Ki-67 mRNA expression for any laboratory.
Molecular information drives treatment choices in breast cancer

- Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and the marker of proliferation Ki-67 are key biomarkers in the evaluation of breast cancer tumors
- The combination of the biomarker results allows the assessment of the different St Gallen subtypes, which are a key parameter for treatment decisions

**Definition of Breast Cancer Surrogate Subtypes (St Gallen 2013)**

<table>
<thead>
<tr>
<th>Breast Cancer Subtypes</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A-like</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>Luminal B-like (HER2 negative)</td>
<td>pos</td>
<td>pos/neg*</td>
<td>neg</td>
<td>pos/neg*</td>
</tr>
<tr>
<td>Luminal B-like (HER2 positive)</td>
<td>pos</td>
<td>pos/neg</td>
<td>pos</td>
<td>pos/neg</td>
</tr>
<tr>
<td>HER2 positive (non-luminal)</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>pos/neg</td>
</tr>
<tr>
<td>Triple negative (ductal)</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>pos/neg</td>
</tr>
</tbody>
</table>

*with the exception of the combination PR pos and Ki-67 neg = Luminal A-like

- Ki-67 is a prognostic and predictive marker. Analytical challenges like high observer variability hinder its standardized and reproducible determination

**MammaTyper®’s RT-qPCR technology has the following accepted advantages:**

- Standardized performance and fast turn-around time
- Minimized inter- and intra-laboratory variability
- Quantitative results with wide dynamic range

**MammaTyper® precisely determines mRNA expression of ER, PR, HER2 and Ki-67**

**MammaTyper® Key Characteristics:**

1. **High-performance test**
   - Quantitative RT-qPCR assay (CE marked IVD)
   - Highly reproducible biomarker assessment
   - Reliable results through standardized biomarker detection
   - Accurate stratification of breast cancer tumors into St Gallen subtypes

2. **Promising clinical utility**
   - Clinical value validated in numerous performance evaluation studies
   - Outperforms IHC by accurate Ki-67 determination
   - Provides information on patient’s prognosis
   - Accurate subtyping supports treatment decisions

3. **Easy-to-use**
   - Reliable method for any molecular pathology laboratory
   - Validated for multiple qPCR instruments*
   - From resection or core needle biopsy FFPE sample to result within 6 hours

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**Noske et al., Comparison of different approaches for assessment of HER2 expression on protein and mRNA level: prediction of chemotherapy response in the neoadjuvant GeparTriO trial. Breast Cancer Res. Treat 2011; 126(1):23-31**

**RT-qPCR = Reverse transcription quantitative real-time PCR**

**Definition of Breast Cancer Surrogate Subtypes (St Gallen 2013)**

- with the exception of the combination PR pos and Ki-67 neg = Luminal A-like

**Clinical value validated**

*Validated RT-qPCR instruments: Roche cobas 4800 Analyzer, Roche LightCycler® 480 I, Applied Biosystems® 7500 Fast Dx, Siemens Versant® kPCR Cycler, Bio-Rad CFX96®, Agilent Technologies Mx3000P

FFPE = formalin fixed paraffin-embedded

**Laible M et al., Technical validation of an RT-qPCR in vitro diagnostic test system for the determination of breast cancer molecular subtypes by quantification of ERBB2, ESR1, PGR and MKI67 mRNA levels from formalin-fixed paraffin-embedded breast tumor specimens. BMC Cancer 2016; DOI: 10.1186/s12885-016-2476-x**

**Wirtz RM et al., Biological subtyping of early breast cancer: a study comparing RT-qPCR with immunohistochemistry. Breast Cancer Res Treat 2016; 157(3), 437-446**


**Sinn HP et al., Comparative analysis of quantitative IHC with automated scoring versus reverse transcription quantitative real-time PCR on FFPE tissue samples for the assessment of ER, PR and Ki-67 labeling index and the prediction of pathological complete response in breast cancer. BMC Cancer 2017; 17:121**
MammaTyper® enhances breast cancer biomarker assessment

MammaTyper® results provide a quantitative measure for each biomarker

Example: Scatter plot shows quantitative MammaTyper® PGR values vs. quantitative IHC PR values

MammaTyper® mRNA expression results correlate very well to quantitative IHC values.

MammaTyper® cut-offs classify biomarker values into positive or negative results. Dichotomized biomarker results enable stratification into subtypes.

Precise and reproducible biomarker assessment with MammaTyper®

- MammaTyper® cut-offs are validated based on clinical outcome
- MammaTyper® results express a wider dynamic range than IHC
- Precise and highly reproducible biomarker results through standardized assessment

Quantitative gene expression report

Results enable accurate molecular subtyping of tumor tissue according to St Gallen guidelines

MammaTyper® provides guidance for treatment decisions of breast cancer patients

Validated cut-offs are used to determine the positivity or negativity of the biomarker

Gene nomenclature: ERBB2 = HER2, ESR1 = ER, PGR = PR, MKI67 = Ki-67
Values in the ranges colored in light blue/orange have been observed in a cohort of 752 breast cancer samples. Values in the ranges colored in dark blue/orange have not yet been observed but are still valid
Varying tumor cell content has minimal influence on MammaTyper® performance*

Fluctuating tumor cell content (TCC) could possibly affect the validity of quantitative assessment of ER, PR, HER2 and Ki-67. Therefore, the performance of MammaTyper® was investigated under different scenarios of TCC.

MammaTyper® results of paired tumor samples with high TCC (> 80 %) derived by macrodissection and low TCC (5 – 50 %) including varying DCIS* content (10 – 70 %)

- Despite varying tumor cell content, MammaTyper® results for HER2, ER, PR and Ki-67 revealed a high level of concordance between low TCC and high TCC samples

MammaTyper® International Multicenter Study demonstrates excellent reproducibility

MammaTyper® International Multicenter Reproducibility Study
has evaluated the inter- and intra-site reproducibility of the quantitative detection of ERBB2, ESR1, PGR and MKI67 mRNA expression in clinical samples
- 10 different sites in Europe, North America and Asia
- Standardized application training
- Locally and centrally extracted total RNA of 24 clinical FFPE samples
- Assessment of the precision of the test under different conditions: Laboratories, operators, instruments, days and lots

MammaTyper® Reproducibility Study results
- Low inter-site variance of quantitative single biomarker results is demonstrated by excellent ICC values ≥ 0.98:
  - Quantitative biomarker results
  - ICC
  - ERBB2 0.987
  - ESR1 0.992
  - PGR 0.998
  - MKI67 0.980

- Excellent inter-site agreement of binary single biomarker classification (positive/ negative) is represented by high Kappa values:
  - Binary biomarker results
  - Kappa values
  - ERBB2 1.00
  - ESR1 0.91
  - PGR 0.94
  - MKI67 0.94

- Determination of breast cancer subtypes shows a high level of agreement across all sites (Kappa = 0.90)

MammaTyper® is highly reproducible – reducing inter- and intra-laboratory variations and allows e.g. standardization of Ki-67 assessment

Wirtz RM et al., Low influence of tumor cell content on mRNA expression levels of ERBB2, ESR1, PGR and MKI67 when performing the MammaTyper® RT-qPCR kit. Poster SABC 2014
Laible M et al., Technical validation of an RT-qPCR in vitro diagnostic test system for the determination of breast cancer molecular subtypes by quantification of ERBB2, ESR1, PGR and MKI67 mRNA levels from formalin-fixed paraffin-embedded breast tumor specimens. BMC Cancer 2016; DOI: 10.1186/s12885-016-2476-x

ICC = Interclass Correlation Coefficient
MammaTyper® is a reliable and accurate test for any molecular pathology laboratory

MammaTyper® Kit Facts
- Designed for 10 determinations (max. 8 patient samples)
- Easy-to-run assay protocol
- Reduced hands-on time for pathologists

MammaTyper® delivers results from FFPE sample to report within 6 hours

Sample preparation
10 µm FFPE tissue section (tumor cell content > 20 %)

RNA extraction
Use of RNXtract® is recommended for RNA extraction.

MammaTyper® test set up
Preparation of mastermixes and distribution on 96 well plate. Analysis of up to 8 patient samples per run.

RT-qPCR analysis
Validated on the following qPCR instruments:
- Roche cobas® 480 Analyzer
- Roche LightCycler® 480 II
- Applied Biosystems® 7500 Fast (Dx)
- Siemens Versant® kPCR Cycler
- Bio-Rad CFX96® (IVD, non-deep well)
- Agilent Technologies Mx3000P

Data processing and reporting
- Export of mRNA expression data
- Calculation and assessment of results
- Results provided within 6 hours

MammaTyper® is an easy-to-use test delivering precise results within 6 hours

MammaTyper® and RNXtract® are registered trademarks of BioNTech Diagnostics GmbH

RNXtract® is an easy to perform and robust procedure for the extraction of highly purified total RNA for RT-qPCR applications like MammaTyper®
MammaTyper® allows accurate biomarker assessment

Clinical performance evaluation: MammaTyper® FinHer study
Concordance of MammaTyper® results with IHC/CISH based standard diagnostic methods was evaluated using 769 tissue samples obtained within the FinHer trial.

Patients:
Node positive or high risk node negative invasive breast cancer.

FinHer trial design:
The trial evaluated the efficacy of combining FEC with Docetaxel vs. Vinorelbin. Patients with HER2 positive tumors were also assigned to receive or not receive Trastuzumab.

Concordance between MammaTyper® and IHC/CISH-based biomarker assessments

<table>
<thead>
<tr>
<th></th>
<th>ESR1 (ER)</th>
<th>PGR (PR)</th>
<th>ERBB2 (HER2)</th>
<th>MKI67 (Ki-67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concorance</td>
<td>91.8%</td>
<td>92.6%</td>
<td>91.8%</td>
<td>75.0%</td>
</tr>
<tr>
<td>PPA</td>
<td>95.9%</td>
<td>93.2%</td>
<td>85.9%</td>
<td>89.1%</td>
</tr>
<tr>
<td>NP</td>
<td>81.7%</td>
<td>89.4%</td>
<td>93.5%</td>
<td>53.7%</td>
</tr>
<tr>
<td>Kappa statistic</td>
<td>0.80</td>
<td>0.68 - 0.85</td>
<td>0.77 - 0.82</td>
<td>0.45 - 0.53</td>
</tr>
<tr>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

PPA Positive percent agreement, NPA Negative percent agreement

Assessment of ER, PR and HER2 by MammaTyper® correlated well with results obtained by IHC and CISH
As expected, Ki-67 shows moderate concordance between the IHC and MammaTyper® results due to known technical limitation in standardization of Ki-67 IHC assessment

MammaTyper® results show a high degree of concordance with IHC/CISH for ER, PR and HER2

MammaTyper® delivers consistent treatment guidance supported by accurate MKI67 determination
Precise Ki-67 evaluation provides prognostic value for patient outcomes

Comparison of Ki-67 expression determined by MammaTyper® and IHC

Favorable DDFS is independently associated with low MKI67 mRNA expression determined by MammaTyper®
Determination of MKI67 by MammaTyper® delivers extended information on patient’s risk of developing distant metastases based on validated cut-off

Clinical outcome proves that Ki-67 determination by MammaTyper® is superior to IHC


Figure modified according to Wirt et al, Breast Cancer Res Treat 2016
DFS = Distant disease free survival
*see page 12
MammaTyper® results ensure precise determination of breast cancer subtypes correlated with clinical outcome

Accurate MKI67 assessment by MammaTyper® has substantial impact on distinction between Luminal A- and B-like breast cancers.

- MammaTyper® luminal subtypes correlated with FinHer® clinical outcome data, thus proving the accuracy of MammaTyper® results

Concordance of molecular surrogate subtypes defined by MammaTyper® and IHC (FinHer® study)

Hypothesis-generating data: MammaTyper® predicts benefit from Taxane treatment* by accurate distinction of molecular subtypes

Patients classified as Luminal B-like (HER2 negative) by MammaTyper® show benefit from Taxane-based chemotherapy.

Comparison of OS and different treatment regimens of Luminal A-like and Luminal B-like (HER2 negative) patients stratified by MammaTyper® or IHC

- Patient stratification based on MammaTyper® shows improved OS of the Luminal B-like (HER2 negative) patients receiving Docetaxel-based treatment compared to those receiving Vinorelbine
- By accurate stratification of patients into Luminal A-like and Luminal B-like (HER2 negative) subtypes using MammaTyper®, patient groups that might benefit from Docetaxel-based treatment can be revealed
- Classification of the patients in Luminal B-like (HER2 negative) subtype by IHC did not reveal a clear separation in responders and non-responders to a Docetaxel-based treatment

By precise subtyping, MammaTyper® results support selection of appropriate treatment strategy for each patient

Hypothesis: MammaTyper® might open up new opportunities of providing predictive information about the benefit of adjuvant Taxane-based treatment

Subtype defined by IHC/CISH

Subtype defined by MammaTyper®

Reclassification of all defined subtypes by MammaTyper®

0.0 0.1 0.2 0.3
0.0 0.1 0.2 0.3

By precise subtyping, MammaTyper® results support selection of appropriate treatment strategy for each patient

Hypothesis: MammaTyper® might open up new opportunities of providing predictive information about the benefit of adjuvant Taxane-based treatment

* FinHer results reclassified according to St Gallen classification

**HR = hazard ratio

Abbreviations

HER2: human epidermal growth factor receptor 2
IHC: immunohistochemistry
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Abbreviations

HER2: human epidermal growth factor receptor 2
IHC: immunohistochemistry

MammaTyper®

Clinical Validation

adjuvant
**Hypothesis-generating data:**

**Predictive value of MammaTyper™ in neoadjuvant setting – first findings**

MammaTyper™ has the potential to predict the probability of neoadjuvant chemotherapy benefit

Proliferative activity of the tumor guides clinical therapy management in primary breast cancer. In the neoadjuvant setting, higher Ki-67 values are consistently associated with higher rates of histologically proven pathological complete response (pCR) to chemotherapy.

**Clinical performance evaluation: MammaTyper™ 1st Neoadjuvant Study**

Concordance between MammaTyper™ and vIHC/qIHC results (FFPE samples from 83 core needle biopsies of patients with primary invasive breast cancer) were evaluated as well as the predictive value of Ki-67 assessment by MammaTyper™ in comparison to IHC.

**Patients:**

Patients with operable (T2-T4/N0-2/M0) breast cancer eligible for neoadjuvant chemotherapy.

**SO80 trial design:**

Patients were randomized to receive sequential Anthracycline/Taxane-based regimens containing either Pemetrexed or Cyclophosphamide in combination with Epirubicin.

The predictive clinical claim is currently under further validation in the MammaTyper™ 2nd and 3rd Neoadjuvant Study.

**Comparison of MammaTyper™ and IHC measurements: Patients responding or not responding to neoadjuvant chemotherapy**

<table>
<thead>
<tr>
<th>Metric</th>
<th>MammaTyper™</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity [%]</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity [%]</td>
<td>52</td>
<td>7</td>
</tr>
</tbody>
</table>

**Clinical Validation**

- Stratification of patients for their probability of achieving a pCR with 100 % sensitivity resulted in a specificity of 52 % using MammaTyper™ MKI67 and a specificity of only 7 % using Ki-67 IHC
- MammaTyper™ MKI67 assessment enables a significant differentiation into groups of responders (pCR) and non-responders (no-pCR) to Taxane-based neoadjuvant chemotherapy
- No significant differentiations of the two groups were possible using IHC for Ki-67 determination
- MammaTyper™ outperforms IHC in predicting pCR in the neoadjuvant setting

**Hypothesis: MammaTyper™ identifies those patients who are likely to benefit from Taxane-based neoadjuvant chemotherapy based on accurate determination of MKI67**

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Sinn HP et al., Comparative analysis of quantitative IHC with automated scoring versus reverse transcription quantitative real-time PCR on FFPE tissue samples for the assessment of ER, PR and Ki-67 labeling index and the prediction of pathological complete response in breast cancer. BMC Cancer 2017; 17:124

Schneeweiss et al., A randomized phase II trial of doxorubicin plus pemetrexed followed by docetaxel versus docetaxel plus cyclophosphamide in combination with Epirubicin. Annals of Oncology 2012; 23:1244

*vIHC = visual immunohistochemistry  *qIHC = quantitative immunohistochemistry
Innovation for your breast cancer diagnostics

**High-performance**
- Precise quantitative results for mRNA expression of HER2, ER, PR and Ki-67
- Outperforms IHC by accurate Ki-67 determination
- Accurate and reliable test to stratify breast cancer into surrogate subtypes acc. to St Gallen

**Reproducible**
- Highly reproducible results with extremely low inter-/intra-laboratory variation

**Validated**
- Clinical value has been validated in numerous performance evaluation studies

**Prognostic**
- Extended information on patient’s prognosis

**Predictive**
- Accurate subtyping supports treatment decisions

**Optimized**
- Reliable method for any pathology laboratory

**Easy-to-use**
- Easy-to-use test which allows results within 6 hours


To order MammaTyper® or for further information, please contact your local distributor: